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The effect of 12 weeks of intermittent resistance training with Algomed supplementation on plasma levels of Fetuin A and Fetuin B in men with obesity

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A B S T R A C T

Introduction: Fetuin A and B are hepatokines linked to obesity and metabolic disorders. The present study aimed to investigate the effect of 12 weeks of intermittent resistance training combined with Algomed (Chlorella vulgaris) supplementation on plasma levels of fetuin A and B in obese men.

Materials & Methods: This quasi-experimental study included 44 obese men (BMI > 30), aged 23–32 years, randomly assigned to four groups (n = 11 each): control, resistance training, Algomed supplementation, and resistance training + Algomed. The training protocol was performed three times per week for 12 weeks. The supplement groups received 1800 mg/day of Algomed. Plasma levels of fetuin A and B were measured by ELISA before and after the intervention. Baseline differences were assessed using one-way ANOVA. Within-group changes were analyzed using paired t-tests, and between-group comparisons of post-test values were conducted using ANCOVA with initial values as covariates. Bonferroni post hoc tests were applied where appropriate ($p < 0.05$).

Results: ANCOVA results showed significant differences in post-test levels of both fetuin A and fetuin B between the groups ($p < 0.0001$). Post hoc analysis indicated that the training + Algomed group had significantly lower fetuin-A levels compared to the control group. Furthermore, fetuin-B levels were significantly reduced in all intervention groups compared to the control, with the greatest reduction observed in the training + Algomed group compared to all other groups ($p < 0.0001$).

Conclusion: Twelve weeks of intermittent resistance training combined with Algomed supplementation had a synergistic effect in reducing plasma levels of fetuin A and B in obese men. These findings suggest potential metabolic benefits for this combined intervention strategy.

Keywords: Resistance Training, Plasma, Men, Obesity

➢ **Cite this paper**
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Introduction

Obesity is one of the most common metabolic disorders in industrialized and developing countries (1). One of the pathological consequences of obesity is the prevalence of cardiovascular diseases and metabolic syndrome. Obesity, problems with blood lipids and their oxidation, high blood sugar, and the wrong amounts of high-density lipoprotein and low-density lipoprotein are all signs of metabolic syndrome. It is one of the main causes of cardiovascular diseases (1). Obesity causes disorders in the system. Obesity negatively impacts various body tissues, including fatty tissue, muscle, and liver (2). The increase in insulin resistance in obese people can occur following the decrease in muscle mass, increase in fatty tissue, and liver disorders; therefore, obesity is considered a fundamental problem for the body's health (3).

Obesity, known as metabolic syndrome, is associated with chronic inflammation (4). Adipose tissue, in addition to being a source of energy storage in the body, produces and releases inflammatory and pro-inflammatory factors (5). Several clinical and experimental studies have shown that people who are overweight or obese have higher levels of inflammatory factors than healthy people. However, when these people lose weight, the levels of these factors go down (6). Several studies have shown an increase in inflammatory factors in the blood of obese people, but the main point is that these people do not show signs of inflammation, and it is also referred to as subclinical inflammation (7). Systemic energy homeostasis is controlled by the liver, which can respond to too little or too much energy by interacting with other tissues. The liver controls systemic energy homeostasis in part by controlling glucose and lipid metabolism and preventing disruptions in these processes. Each of these processes can lead to metabolic disorders and be effective in the development of insulin resistance or fatty liver disease (6). In recent years, some elements that are exclusively or mainly secreted by liver tissue, such as hepatokines, have been noticed as molecules with strong metabolic effects (8). Some researchers have also found hepatokines to be a link between obesity and cardiovascular diseases. The levels of some hepatokines have been shown to rise and fall in people who are overweight or have cardiovascular diseases (8). Fetuin A and Fetuin B are among the well-known hepatokines on which many studies have been conducted. Fetuin A is known as a glycoprotein that is mainly secreted by liver tissue, and its high levels have been observed in the blood circulation (9). In humans, fetuin-A has been located on chromosome 3q27 as a susceptibility locus for metabolic syndrome and type 2 diabetes mellitus (10). It is well known that there is a strong link between fetuin-A levels and the risk of developing type 2 diabetes. However, fetuin-A has many other effects on the body besides just this one organ (11).

Researchers have found that fetuin B and fetuin A share 22% of their genetic material. Like fetuin A, fetuin B is a plasma protein that comes from the liver (hepatokine). (21) Upregulation of serum fetuin A levels when increasing liver fat levels and non-alcoholic fatty liver disease (NAFLD) has been observed in adults (16). Heart patients with higher fetuin A levels are also four times more likely to have a myocardial infarction or an ischemic stroke than those with low fetuin A levels. Also, the evidence shows that women are much more likely to have heart diseases when their fetuin-A levels are high. There is a comparison with men (17), which shows the different effects of fetuin A depending on the gender of people. An increase in fetuin A levels has been reported in patients with fatty liver independent of the amount of fat mass (18). Several things cause this hepatokine to be expressed higher. One of these is that FAA levels raise the activity of NF-κB, which in turn raises the expression of fetuin A (19). Some researchers have also reported that high levels of glucose can increase the expression of fetuin A. Some researchers have also found that high glucose levels raise the expression of fetuin A by turning on the ERK-1 and ERK-2 signaling pathways (20). Protein kinase 1 (ERK-1) and mitogen-activated protein

kinase 2 in B levels have been observed in patients with non-alcoholic fatty liver compared to healthy controls, which emphasizes the pathological effects of this hepatokine in the occurrence of fatty liver disease (23).

The use of herbal supplements or sports supplements during sports activities enhances energy regeneration after exercise, increases endurance under pressure, or helps the body return to its initial effective state. These supplements are specifically designed for use after training. Algomed Algae, with the scientific name of Chlorella vulgaris, is the main component and effective ingredient of this algae. It possesses antioxidant and anti-inflammatory properties (17). Chlorella vulgaris has been confirmed for having anti-tumor, antioxidant, anti-inflammatory, and antimicrobial activities. Conversely, it has been observed that chlorella lowers blood pressure and cholesterol levels, accelerates wound healing, and strengthens the body's immune system. In addition, chlorella strongly suppresses low-density lipoprotein (LDL) cholesterol levels (18).

However, there is still no information regarding the effect of Chlorella vulgaris supplements, including in combination with exercise training, on the levels of different hepatokines as important mediators for the positive effects of exercise training, necessitating further investigation. changes in the levels. Several hepatokines are released after exercise and Algomed supplementation. These include hepatokines whose effects and mechanisms are still mostly unknown, which is not a secret to anyone. The researcher is left with limited and conflicting information in this area. So, the point of this study was to find out what happened to fetuin A and fetuin B levels in overweight men who did intermittent resistance training with algae supplements for 12 weeks.

Materials and methods

Setting and sample

TThe current research was a quasi-experimental study with a pre-test-post-test design. From the statistical population of obese men aged 23 to 32, 44 eligible volunteers were selected as subjects in a targeted and available manner. The criteria for entering the research: the conditions for entering the implementation of the research are no drug and alcohol addiction, no history of regular sports activity for at least 6 months, no history of kidney or liver disease or diabetes, having a condition (Body Mass Index more than 30), and not having any injury or physical problem for the subjects, and they were included in the study after being examined by a cardiologist. Exclusion criteria include being a smoker, consuming alcohol, having an allergy to the Algomed supplement, causing injury during exercise, and causing disease during training; he was withdrawn from the study.

Sample Size, Randomization and Blinding

The subjects were randomly divided by lottery into four groups: intermittent resistance training (11 men), Algomed supplement (11 men), intermittent resistance training + Algomed supplement (11 men), and control (11 men).

Measurements & Validity and Reliability

1.Demographic tool

Demographic variables include age, marital status, education level, occupation, economic status, family medical history, personal medical history, and daily physical activity level.

2. Blood Sample Analyzing

The first blood sample was taken fasting 48 hours before, and the second blood sample was taken 48 hours after the twelve-week training period from the right brachial vein of the subjects. The blood samples taken were transferred to special test tubes for plasma preparation. (tubes containing EDTA). The resulting plasma was kept at -70 degrees Celsius. All test stages were conducted at 8–10 a.m. in standard conditions. Fetuin A and fetuin B were measured using an ELISA device and kit (Trinity Biotech USA, St. Louis, MO).

Intervention

The training, exercise, and supplement group trained according to the schedule for twelve weeks. Each training session started with 10 minutes of general warm-up (slow running, stretching, and softening), 3-5 minutes of special warm-up, and then the main program of periodic resistance exercises for the groups. Muscular consisted of 8 movements of the upper body and lower body (squat, chest press, knee bend, forearm press, leg press, barbell overhead, back of the machine, and underarm cable pull from the back), which Figure 3 is a set of 13 repetitions with 60 The percentage of 1RM and rest between active sets was performed with an intensity of 20% of 1RM and 15 repetitions, and at the end, they finished with a 10-minute cool-down. The principle of overload means that 5-10% of 1RM was added to their weight every 2 weeks. One repetition maximum (1RM: one repetition maximum) of the subjects was calculated using the Berzyski equation (19). It can be done if the weight is light and the number of repetitions is more than 10; after a little rest, a heavier weight is chosen until it can do less than 10 repetitions. The amount of weight and the number of repetitions in Each movement is recorded and then put into the formula. One maximum repetition = weight moved (kg) / 1.0278 - number of repetitions until fatigue (0.0278) Simultaneously with the intermittent exercise group and the supplement group, 1800 mg The supplement was administered in the form of 6 tablets with a glass of water (2 tablets an hour and a half before breakfast, 2 tablets an hour and a half before lunch, and 2 tablets an hour and a half before dinner). The factory's instructions were used. The placebo group also took starch tablets of the same color as Algomed tablets. The control group also used a placebo during this period. It should be noted that food notes were taken from all the subjects three days before the pre-test blood sampling and three days before the post-test blood sampling. Subjects went to the laboratory on the morning of the test day from 8 to 10 am fasting to measure their body composition. Body weight was measured with a digital scale (Seka model, made in Germany, accurate to ±0.5 kg) while the person was standing by a wall without shoes and wearing only the bare necessities. Height was measured with a wall height gauge (model 44440, made by the Kaveh company, Iran, accurate to ±0.1 cm) while the person was standing by the wall without shoes and with their shoulders relaxed.

Ethical consideration

Ethical considerations consisted of obtaining an ethics code (IR.SSRC.REC.1402.161), honesty in library collection and data report, and written informed consent from all samples according to the Declaration of Helsinki's announcement and interventional human principles.

Statistical and Data Analysis

Descriptive statistics (mean ± standard deviation) were used to summarize demographic characteristics and outcome variables. The Shapiro-Wilk test was performed to assess the normality of data distribution for each variable. To compare baseline demographic variables between the four groups, a one-way analysis of variance (ANOVA) was performed to evaluate within-group differences between pre-test and post-test scores, and a paired sample t-test was conducted. For between-group comparisons of post-test outcomes, a one-way analysis of covariance (ANCOVA) was used, with pre-test values included as covariates to control for baseline differences. When ANCOVA showed significant effects, Bonferroni post hoc tests were applied to determine pairwise group differences. All statistical analyses were conducted using SPSS software version 16. A p-value of less than 0.05 was considered statistically significant.

Results

In order to examine the distribution of data, the Shapiro-Wilk test was used, and the results of this test indicated a normal distribution of the research data ($P > 0.05$). The results of the Shapiro-Wilk test for the

variables examined in the present study are reported in Table 1.

Table 1. Description of the Shapiro-Wilk test.

Variables	Statistics	P Value (Shapiro-Wilk)
Fetuin A(Pg/ml)	0.960	0.125
Fetuin B(Pg/ml)	0.958	0.113
BMI (Kg/m2)	0.604	0.859
weight (kg)	0.738	0.738
Percent Body Fat	0.701	0.709

The demographic characteristics of the subjects at the pretest stage (mean ± standard deviation) are described (Table 2).

Table 2. Demographic characteristics of the participants.

Variable/Group	Control	Algomed + Intermittent Resistance	Intermittent resistance	Algomed	P -Value (Anova)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Number	11	11	11	11	-
Age (years)	30.0±2.3	24.9±3.6	29.3±2.4	26.4±2.6	0.0002
weight (kg)	97.0±5.7	96.5±5.2	100.7±8.0	92.4±7.4	0.0526
Height (cm)	173.8±3.7	173.1±4.0	176.7±4.8	170.2±5.4	0.0177
BMI (Kg/m2)	32.0±1.3	31.9±1.7	32.5±1.7	32.2±1.6	0.8492
Percent Body Fat	34.3±2.0	31.0±1.1	33.0±2.0	34.7±2.4	0.0002

Within-group comparisons using paired t-tests showed that Fetuin A levels significantly decreased only in the Exercise + Algomed group ($p = 0.001$), while no statistically significant changes were observed in the control, Algomed-only, or exercise-only groups ($p > 0.05$). In contrast, fetuin B levels decreased significantly in all intervention groups compared to their own pre-test values, including the Algomed-only ($p = 0.008$), Exercise-only ($p = 0.003$), and Exercise + Algomed ($p < 0.001$) groups. No significant change in fetuin B was found in the control group ($p = 0.918$). (Table 3).

Table 3. The results of within-group comparisons (Paired t-test) of Fetuin A and B levels.

Group	Fetuin A (Pre-test)	Fetuin A (Post-test)	p-value	Fetuin B (Pre-test)	Fetuin B (Post-test)	p - value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Control	4.91 ± 0.09	4.90 ± 0.10	0.732	5.10 ± 0.08	5.09 ± 0.09	0.918
Algomed + Intermittent Resistance	4.80 ± 0.08	3.90 ± 0.07	0.001	5.00 ± 0.10	4.20 ± 0.10	0.001

Intermittent resistance	4.85 ± 0.07	4.20 ± 0.08	0.094	5.05 ± 0.09	4.50 ± 0.11	0.003
Algomed	4.95 ± 0.10	4.80 ± 0.11	0.086	5.10 ± 0.10	4.60 ± 0.12	0.008

ANCOVA revealed significant differences between groups in post-test levels of Fetuin A ($F = 127.64$, $p < 0.0001$) and Fetuin B ($F = 152.96$, $p < 0.0001$). Bonferroni post hoc tests showed that: Fetuin A: Only the Exercise + Algomed group had significantly lower post-test levels compared to the control ($p = 0.001$). Fetuin B: All intervention groups (Algomed, Exercise, and Exercise + Algomed) had significantly lower post-test values than the control group ($p < 0.01$), with the Exercise + Algomed group showing the greatest reduction compared to all other groups ($p < 0.001$). These results suggest a synergistic effect of combining intermittent resistance training with Algomed supplementation on reducing plasma levels of fetuin A and B. (Table 4).

Table 4. Results of covariance analysis for fetuin A and fetuin B levels.

Variable	Group	df	Mean square	F	P Value
Fetuin A (Pg/ml)	Corrected model	4	--------	127.64	0.0001
	Initial values	1	0.0096	0.99	0.3269
	Group	3	1.2478	127.64	0.0001
	Error	39	0.0098	------	--------
	Total	44	-------	------	--------
Fetuin B (Pg/ml)	Corrected model	4	-------	152.96	0.0001
	Initial values	1	0.0048	0.57	0.4567
	Group	3	1.3095	152.96	0.0001
	Error	39	0.0086	-------	-------
	Total	44	-------	-------	-------

Based on the Bonferroni post hoc test results, a significant reduction in fetuin A levels was observed only in the Exercise + Algomed group compared to the control group ($p = 0.030$). No other pairwise differences in fetuin A were statistically significant ($p > 0.05$). Regarding Fetuin B levels, all intervention groups (Algomed, Exercise, and Exercise + Algomed) showed significantly lower post-test values compared to the control group ($p < 0.01$). Furthermore, the Exercise + Algomed group showed a significantly greater reduction in Fetuin B levels compared to both the Algomed and Exercise-only groups ($p < 0.001$). Pairwise comparisons of post-test fetuin B levels between groups using the Bonferroni post hoc test are presented in Table 5.

Table 5. The results of post hoc Bonferroni test for fetuin A and fetuin B levels.

Variable	Group	Group	Mean df	P Value
Fetuin A (Pg/ml)	Control	Algomed	1.283	0.307
		Intermittent Resistance	1.520	0.138
		Algomed+Intermittent Resistance	1.906	0.30

	Algomed	Control	1.283	0.307
		Intermittent Resistance	0.238	1.000
		Algomed+Intermittent Resistance	0.624	1.000
	Intermittent Resistance	Control	1.520	0.138
		Algomed	0.238	1.000
		Algomed+Intermittent Resistance	0.386	1.000
	Algomed+Intermittent Resistance	Control	1.906	0.030
		Algomed	0.624	1.000
		Intermittent Resistance	0.386	1.000
Fetuin B (Pg/ml)	Control	Algomed	0201	0.001
		Intermittent Resistance	0.267	0.001
		Algomed+Intermittent Resistance	0.308	0.001
	Algomed	Control	0.201	0.001
		Intermittent Resistance	0.065	0.001
		Algomed+Intermittent Resistance	0.106	0.001
	Intermittent Resistance	Control	0.267	0.001
		Algomed	0.065	0.001
		Algomed+Intermittent Resistance	0.041	0.001
	Algomed+Intermittent Resistance	Control	0.308	0.001
		Algomed	0.106	0.001
		Intermittent Resistance	0.041	0.001

Discussion

The purpose of this study was to investigate the effect of 12 weeks of intermittent resistance training with an Algomed algae supplement on the levels of selected hepatokines, such as fetuin A and fetuin B, in obese men. The main result of this study was that fetuin A levels went down after 12 weeks of intermittent resistance training and Algomed supplementation. However, this hepatokine drop was only significant in the training + Algomed group. Also, fetuin B levels went down significantly after different interventions (exercise, Algomed, and exercise + Algomed). The biggest drop in fetuin B levels was seen in the exercise + Algomed group, showing that the drop in fetuin B levels was also significant compared to the Algomed and exercise groups. These results demonstrate that combining Algomed use with intermittent resistance training has a synergistic effect on lowering fetuin A and fetuin B levels.

Upregulation of fetuin-A levels has been shown in obesity and related disorders, such as metabolic syndrome, type 2 diabetes, and myocardial infarction/stroke (15, 16, 17). That being said, this study showed that intermittent resistance training, both by itself and especially when combined with Algomed, was a good way to lower fetuin A and fetuin B levels. In previous studies, the role of exercise training in reducing fetuin A (18) and fetuin B (19) levels has been shown, and the decrease in the levels of these two hepatokines was associated with the improvement of metabolic status, including

insulin resistance and lipid profiles (16, 17). The researchers confirmed the current findings in their study, stating that sports training at varying intensities results in a decrease in fetuin A levels, even when two types of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) were used. The researchers found no difference between the two exercise training programs in reducing fetuin A levels. According to these researchers, regular exercise may weaken the main stimulus for fetuin A's initial rise, leading to its decrease (21). This can happen because exercise lowers the amount of fat in the liver and, in turn, lowers the toxic effects of sugar on fat in the liver. Digrov Kihanian et al. did a study on type 2 diabetic men and found that aerobic and resistance training significantly lowered fetuin A and B levels. These results are similar to what we found here. They also found that eight weeks of resistance training was more effective than aerobic training at lowering fetuin A and B levels. The levels of fetuin A (18.3% vs. 7.9%) and fetuin B (29.2% vs. 11.45%) are more effective. These researchers found that decreased levels of fetuin A and fetuin B were associated with a decrease in insulin resistance (22). Even so, researchers have found that fetuin A levels drop after eight weeks of intense interval training in overweight women. This effect is independent of changes in metabolic profile, such as insulin resistance, as well as changes in body composition (23). In a different study, levels of both fetuin A and B dropped significantly in diabetic men who did both resistance and aerobic exercise for 12 weeks. It was found that lowering fetuin A levels and raising plasma and liver lipid levels were linked to stopping pro-inflammatory mediators and turning on Akt (24). Unfortunately, the examination of these paths was beyond the goals of the present study. Researchers are still looking into the cellular and molecular processes that lower fetuin-A levels with exercise. However, they have found that one possible way that fetuin A levels may go down with exercise is by increasing PPAR-γ, decreasing glucolipotoxicity by changing reactive oxygen species (ROS), and activating protein kinase B, which is also known as Akt. Although these examples have been given, more research needs to be done to find out how other sports activities work to lower ferin A and B levels.

In addition to the positive effects that exercise training had on changing the levels of hepatokines that were looked at in this study, the results of the next study will show how the Algomed supplement alone can lower fetuin B and fetuin A levels and how it can make exercise training more effective. It was in lowering the amounts of fetuin A and fetuin B, which shows how Algomed consumption works together to lower the amounts of fetuin A and B. It has been proven that some food constituents play an important role in preventing obesity and related disorders. For example, chlorella is a type of nutrient that consists of single-celled green algae that are rich in chlorophyll, carotenoids, vitamins, minerals, and proteins. It is used as a supplement to maintain health. Gird (26) and the effects of this supplement in preventing some disorders, such as high blood pressure (27), hyperlipidemia, atherosclerosis, and suppression of immunopharmacological effects (28), have been shown. In addition, the anti-hyperinsulinemic effects of chlorella have been observed, and these positive effects are attributed to its role in modulating adipose tissue hypertrophy and the release of adipocytokines. Thus, the downregulation of leptin and MCP-1 levels as inflammatory cytokines and the upregulation of adiponectin levels as anti-inflammatory cytokines were observed following chlorella supplementation (28).

There is no information on what happens to the levels of the variables being studied when you eat algal algae and work out at the same time. However, after eight weeks of aerobic exercise alone, researchers found a significant drop in leptin and LDL levels and an equally significant rise in HDL levels. A study combined eating Chlorella vulgaris algae with exercise and showed that exercise lowers leptin and lipid profile, lowers body weight, and improves lipid

oxidation. Chlorella vulgaris has these benefits because it has a high concentration of niacin, and it also has a lot of fiber, glycolipids, and phospholipids, which all act together to lower lipids (29). In addition, researchers have shown that intense intermittent exercise along with the consumption of Chlorella vulgaris can play a major role in improving the lipid profile and glycemic status of overweight and obese women (30). As proof that Chlorella vulgaris can help the body use glucose better, this supplement has been shown to stop insulin resistance in animals that have been fed a high-fat diet. This was shown by making the animals' glucose and insulin tolerance higher. The good things about chlorella were found to be because it improved the insulin signaling pathway by making more phosphorylation of the insulin receptor (IR), IRS-1, and Akt (31).

It has also been shown that giving rats chlorella supplements along with aerobic exercise can improve the PI3K, Akt, and GLUT-4 signaling pathways in their muscles compared to giving them nutrients alone. Chlorella by itself or aerobic exercise can stimulate PI3K, Akt, and GLUT4, so taking chlorella along with exercise may help stimulate themeven more. This can help people with hyperglycemia better manage their condition. becomes type 2 diabetic (32). In this way, researchers looked at overweight men and found that six weeks of intense intermittent exercise and chlorella consumption improved insulin resistance and lowered IL-6 levels. The group that did training plus chlorella had lower insulin resistance and significantly higher IL-6 levels than the group that did training without chlorella. This shows that chlorella enhances the anti-inflammatory effects of exercise (33).

Limitations of the Study

This study has There are several limitations, including a lack of precise control over the subjects, routine nutrition, and limited information on the simultaneous effects of exercise training and Algomed. Additionally, due to the availability of male samples, the research was conducted exclusively in the male population within the age range of 23 to 32 years.

Conclusion

The current study showed that taking Algomed by itself can effectively lower the levels of hepatokines in overweight men. When combined with exercise, this supplement was even more effective at lowering the levels of hepatokines. According to the present findings, it can be concluded that taking Algomed is an effective nutritional supplement to strengthen the positive effects of exercise, and adding it to the exercise program of obese men can have a synergistic effect. It is recommended that future research examine different types. In measuring body composition, more accurate methods such as DEXA should be used. The measurement of antioxidant indices, liver factors, and inflammatory factors should be investigated.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' contributions

Conceptualization, Methodology, Data Curation, Writing– Review & Editing, Project Administration: MMM, HAN, Validation: MGH, Formal Analysis, Supervision: FGH, HAN, Investigation, Writing– Original Draft Preparation, Visualization MMM, HAN, FGH, Resources: MMM.MGH, Funding Acquisition: No fund.

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